

# Hanging Drop Vapor Diffusion Crystallization

## Crystal Growth 101

The hanging drop vapor diffusion technique is the most popular method for the crystallization of macromolecules. The principle of vapor diffusion is straightforward. A drop composed of a mixture of sample and reagent is placed in vapor equilibration with a liquid reservoir of reagent. Typically the drop contains a lower reagent concentration than the reservoir. To achieve equilibrium, water vapor leaves the drop and eventually ends up in the reservoir. As water leaves the drop, the sample undergoes an increase in relative supersaturation. Both the sample and reagent increase in concentration as water leaves the drop for the reservoir. Equilibration is reached when the reagent concentration in the drop is approximately the same as that in the reservoir.

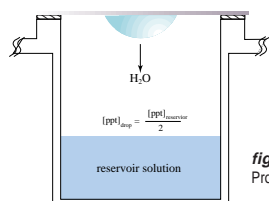


figure 1  
Process of vapor diffusion.

### Benefits of Hanging Drop Crystallization

- Can be cost effective.
- Sample and reagents in contact with a siliconized glass surface.
- Relatively easy access to crystals.
- Can perform multiple drops (experiments) with a single reservoir.

### Using the VDX Plate

The VDX Plate is a 24 well plate manufactured from clear polystyrene. The VDX Plate is typically sealed with High Vacuum Grease (HR3-510) and Siliconized 22 mm Circle or Square Glass Cover Slides. The VDX Plate is also available pregreased. Rows of the plate are labeled A-D and columns are labeled 1-6 on the VDX Plate.

1. Apply a bead of High Vacuum Grease along the top edge of the raised reservoir A1 of the VDX Plate. It is recommended that one apply the high vacuum grease prior to pipetting the reagent. High vacuum grease may be applied by using the Grease Kit (HR3-506). Create a circular bead on the upper edge of the reservoir. Do not complete the circle. Leave a 2 mm opening between the start and finish of the circular bead. Apply the cover slide, press to relieve the air pressure and twist to close the gap. One may also use the VDX Plate Greased. These plates come pregreased.

2. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the VDX Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)

3. Clean a Siliconized 22 mm Circle or Square Cover Slide by wiping the cover slide with lens paper and blowing the cover slide with clean, dry compressed air. Pipet 1 microliter of sample into the center of a Siliconized 22 mm Circle or Square Cover Slide. (Note: Recommended total drop volume is 1 to 40 microliters)

### figure 2



4. Pipet 1 microliter of reagent from reservoir A1 into the drop on the cover slide containing the sample. (Note: Some people prefer to mix the drop while others do not. Proponents of mixing leave the pipet tip in the drop while gently aspirating and dispensing the drop with the pipet. Mixing ensures a homogeneous drop and consistency drop to drop. Proponents of not mixing the drop simply pipet the reagent into the sample with no further mixing).

5. Holding the cover slide with forceps, the Pen Vac, or on the edge between your thumb and forefinger, carefully yet with out delay invert the cover slide so the drop is hanging from the cover slide.

6. Position the cover slide onto the bead of grease on reservoir A1. Gently press the slide down onto the grease and twist the slide 45° to ensure a complete seal.

7. Repeat for reservoir 2 through 24.

### VDX Plate Tips

- Note the VDX Plate has a raised cover to protect the cover slides during transport and storage.
- To access a drop and/or reservoir simply grasp the edge of the cover slide with forceps or fingertips, twist and pull gently.
- VDX Plates can be stacked for convenient storage.
- One can pipet multiple drops onto the cover slide. This technique is often useful when screening additives since one can use the same reservoir with multiple drops with each drop containing a different additive. This technique can also be used to screen different drop sizes and ratios versus the same reservoir. Use care not to avoid mixing the drops during pipetting, plate transport, and plate viewing.

### Using the Q Plate

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the Q Plate. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)

2. Clean a Siliconized 18 or 22 mm Circle Cover Slide by wiping the cover slide with lens paper and blowing the cover slide with clean, dry compressed air. Pipet 2 microliters of sample into the center of a Siliconized 18 or 22 mm Circle Cover Slide. (Note: Recommended total drop volume is 1 to 40 microliters)

3. Pipet 2 microliters of reagent from reservoir A1 into the drop on the Cover Slide containing the sample. (Note: Some people prefer to mix the drop while others do not. Proponents of mixing leave the pipet tip in the drop while gently aspirating and dispensing the drop with the pipet. Mixing ensures a homogeneous drop and consistency drop to drop. Proponents of not mixing the drop simply pipet the reagent into the sample with no further mixing).

4. Holding the cover slide with forceps, Pen Vac, or on the edge between your thumb and forefinger, carefully yet with out delay invert the cover slide so the drop is hanging from the cover slide.

5. Place the siliconized cover slide with the drop onto the step inside reservoir A1 of the Q Plate. (Note: An 18 mm cover slide rests on the lower step while a 22 mm cover slide rests on the upper step. Since the 18 mm cover slide is closer to the reservoir, vapor equilibration occurs at a higher rate than when using 22 mm cover slides).

6. Repeat steps 1 through 5 for the remaining 23 reservoirs.

7. Seal the Q Plate plate with 3 strips of Clear Sealing Tape (HR4-510).

### Using the Q Plate II

1. Pipet 1.0 milliliter of crystallization reagent into reservoir A1 of the Q Plate II. (Note: Recommended reservoir volume is 0.5 to 1.0 milliliters)

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2. Clean a Siliconized 18 mm Circle Cover Slide by wiping the cover slide with lens paper and blowing the cover slide with clean, dry compressed air. Pipet 2 microliters of sample into the center of a Siliconized 18 mm Circle Cover Slide. (*Note: Recommended total drop volume is 1 to 40 microliters*)

3. Pipet 2 microliters of reagent from reservoir A1 into the drop on the Cover Slide containing the sample. (*Note: Some people prefer to mix the drop while others do not. Proponents of mixing leave the pipet tip in the drop while gently aspirating and dispensing the drop with the pipet. Mixing ensures a homogeneous drop and consistency drop to drop. Proponents of not mixing the drop simply pipet the reagent into the sample with no further mixing.*)

4. Holding the cover slide with forceps, Pen Vac, or on the edge between your thumb and forefinger, carefully yet with out delay invert the cover slide so the drop is hanging from the cover slide.

5. Place the siliconized cover slide with the drop onto the ledge inside reservoir A1 of the Q Plate II.

6. Repeat steps 1 through 5 for the remaining 23 reservoirs.

7. Seal the Q Plate II plate with 2 strips of Clear Sealing Tape (HR4-510).

### Q Plate & Q Plate II Tips

- Use Crystal Clear Sealing Tape (HR4-510). Other Brands are optically inferior and the adhesive will turn opaque with certain crystallization reagents.
- To access a drop and/or reservoir of a Q Plate or Q Plate II sealed with tape simply make a circular incision in the tape using the inside of the reservoir as a guide. Use a sharp blade to cut the tape and hold the incised piece of tape with forceps. The opening can be sealed with another strip of tape.
- One can pipet multiple drops onto the cover slide. This technique is often useful when screening additives since one can use the same reservoir with multiple drops with each drop containing a different additive. This technique can also be used to screen different drop sizes and ratios versus the same reservoir. Use care not to avoid mixing the drops during pipetting, plate transport, and plate viewing.
- The Q Plate can also be used for hanging drop and sandwich drop vapor diffusion experiments. The Q Plate II can also be used for hanging drop vapor diffusion experiments.
- Use care when transporting and viewing Q Plate and Q Plate II's. A bump to the plate can toss the cover slide out of position or onto the tape. In very dry, high static environments one may prefer to treat the plates and slides with a static removing device to prevent the glass slides from "jumping" onto the tape.

### Other Plates

The Linbro® Plate and the Costar™ 3424 Plate are also used for hanging drop vapor diffusion crystallization.

### Technical Support

Inquiries regarding the hanging drop crystallization method, interpretation of screen results, optimization strategies and general inquiries regarding crystallization are welcome. Please e-mail, fax, or telephone your request to Hampton Research. Fax and e-mail Technical Support are available 24 hours a day. Telephone technical support is available 8:00 a.m. to 5:00 p.m. USA Pacific Standard Time.

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